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TITLE: A Model of Penetrating Traumatic Brain Injury Using an Air Inflation Technique

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THIS TECHNICAL REPORT HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION.

Frank Tortella
This report describes a method for modeling penetrating traumatic brain injury (PTBI) caused by gunshot using rats as the subjects. The method will be an improvement over previously used techniques in that it is minimally invasive, humane and based on a mathematical approach that is founded on known ballistic biophysics. This technique specifically avoids using a fired projectile. The proposed use of this animal model should improve the understanding of the pathophysiology of penetrating traumatic brain injury.
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Introduction

The objective of this study is to develop a method for modelling penetrating traumatic brain injury (PTBI) caused by gunshot using rats as the subjects. The method will be an improvement over previously used techniques in that it is minimally invasive, humane and based on a mathematical approach that is founded on known ballistic biophysics. This technique specifically avoids using a fired projectile. The proposed use of this animal model should improve the understanding of the pathophysiology of penetrating traumatic brain injury.

The initial objective is to develop a mathematical model for rats that describes the biophysics of wound formation following a ballistic injury. The bullet-size model will be the full metal jacket ammunition like the 7.62mm round and the 9mm which are most commonly used in the military. These equations can then be used generically to determine the dimensions of the salient features of ballistic injury, i.e. the size and shape of the permanent and temporary wound cavities, rapidity of expansion and duration of inflation.

The second objective is to develop a device for minimal invasive recreation of the wound in rats. This device will be based on an air inflation technique. It will be built exactly to precise specifications based on the above mathematical model. This devise will be designed in accordance with all elements of the wound arising from a gunshot. The wound cavities assume the proper size, shape and for the appropriate duration.

The third objective will be to conduct in vivo validation study in rats to demonstrate that this model can recreate wounds similar to those caused by military gunshot. Histopathologic findings will be compared to the results from human gunshot victim autopsy reports and to the work of Carey et al (1989, 1990) using the now-abandoned fired projectile feline model.

This report contains the results of all of the above objectives.

Construction of the mathematical model

In the development of the mathematical model it is important to understand the flight characteristics of a projectile. The penetrating model consists of two distinct phases during its flight. These are the stable and the unstable phases of flight in a representative medium, like gelatin. The portion of a projectile's stable flight is a function of a number of variables, such as initial velocity, the shape of the projectile (drag characteristics), rotational velocity (spin stabilization) and the medium (μ) of travel. These are a few of the major parameters that come into play, and do not neglect the usual forces that act on the projectile such as gravity. Ammunition producers go through great pains to insure flight stability over very wide ranges of operation. Ballistic flight dynamics are well documented, Sellier and Kneubuehl's text (1994) as well as Bellamy and Zajtchuk's treatment in the Textbook of Military Medicine (chapter 4) both provide an excellent review of this subject. During stable flight energy transmitted (dissipated) into the medium is a minimum because the manufacturers have designed the projectile for maximally effective ranges and thereby minimizing the drag on the projectile. During unstable flight a maximal amount of energy is dissipated into its medium. This is the phase of flight we are focused on because it generates the large temporary cavity which causes the most damage. The key point underlying this study is that the center of pressure (CP) is not the same as the center of gravity (CG) of the projectile and as the projectile continues its path in the medium be it air, soap, gelatin or brain tissue it will eventually become unstable and tumble. The instability is due primary to drag forces on the projectile, along with the existence of a "yaw angle" (aerodynamically "angle of attack"). The combined effect of drag forces and small yaw angles eventually causes the CP to move, and in turn the projectile becomes unstable and tumbles.
During the tumbling action more energy will be dissipated into its surrounding medium. This will accentuate the production of a larger temporary cavity. The energy dissipated is modelled as an ellipsoid, where the major and minor axis governing its shape is related to the energy and associated velocity.

The model for describing the formation of the temporary cavity and its relation to the energy levels are described below, assuming the projectile remains intact after penetration. This assumption allows for the mass to remain a constant.

The energy available and amount of energy dissipate in its medium is characterized in figure 1.0, below where:

- $E_0$ is the muzzle energy, and $V_0$ its associated muzzle velocity.
- $E_a$ is the energy dissipated during free flight, which is a function of range to target and projectile design.
- $E_1$ is the energy available at impact, and $V_1$ the associated velocity.
- $E_b$ is energy dissipated on impact, and $V_b$ the associated velocity.
- $E_i$ is the initial impact energy available in the medium ($\mu$) to form the temporary and permanent cavities.
- $E_c$ is the energy dissipated in the medium ($\mu$), while forming of the temporary cavity during its stable flight, and $V_c$ is the associated velocity.
- $E_2$ is the energy available in the medium to form the large temporary cavity during the unstable flight, and $V_2$ is the associated velocity.
- $E_d$ is the energy dissipated in the medium ($\mu$), while forming the temporary cavity during unstable flight or deformation of the projectile. $V_d$ is the associated velocity.
- $E_r$ is the residual energy, and $V_r$ is the associated velocity.

![Figure 1.0 Energy Profile for Intact Projectiles](image)

Figure 1.0 Energy Profile for Intact Projectiles

- $E_0$ is the muzzle energy, and $V_0$ its associated muzzle velocity.
- $E_a$ is the energy dissipated during free flight, which is a function of range to target and projectile design.
- $E_1$ is the energy available at impact, and $V_1$ is the associated velocity.
- $E_b$ is energy dissipated on impact, and $V_b$ is the associated velocity.
- $E_i$ is the initial impact energy available in the medium ($\mu$) to form the temporary and permanent cavities.
- $E_c$ is the energy dissipated in the medium ($\mu$), while forming of the temporary cavity during its stable flight, and $V_c$ is the associated velocity.
- $E_2$ is the energy available in the medium to form the large temporary cavity during the unstable flight, and $V_2$ is the associated velocity.
- $E_d$ is the energy dissipated in the medium ($\mu$), while forming the temporary cavity during unstable flight or deformation of the projectile. $V_d$ is the associated velocity.
- $E_r$ is the residual energy, and $V_r$ is the associated velocity.

The muzzle energy and velocities of all ammunition projectiles are well documented and have been shown to vary considerably. However for the purposes of this study a set of Firing Tables are provided in Sellier and Kneubuehl text (pages 356 to 373) and these data will be used as the basis of free flight performance (air). It measured data but represents typical manufacturers' data. The projectiles under consideration are the 7.62 mm NATO round characterized by 9.5g
mass, muzzle velocity of 830 m/s and the 9mm parabellum round of 8g mass and muzzle velocity of 350 m/s. The 7.62 x39mm AK-47 round of 8 g mass and muzzle velocity of 716 m/s was also investigated. These were selected since they are very common military rounds and widely used in all arenas, including the 9mm for civilian law enforcement. The performance of 7.62mm NATO round will be representative of supersonic projectiles and the 9mm parabellum will represent the transonic or low velocity projectiles. Note that the muzzle velocity of the 9mm round is transonic but within 20 meters from the muzzle its velocity has dropped sufficiently so that upon impact it maybe considered subsonic.

The equations describing the energy and associated velocity are all related back to the muzzle energy (Eo) and muzzle velocity (Vo), with the fundamental assumption that the mass of the projectile is constant. The projectile may tumble after entering the brain or may deform (hollow point) but as long as the projectile does not fragment the mass will be constant. E1 is the energy available at impact and V1 is its associated velocity after a period of free flight. The velocity at impact (V1) is derived by:

\[ V1 = Vo - (Br) \times (\text{Range to Target from muzzle}) \]  

(equation 1.0)

where the constant (Br) is a derived constant, based on free flight data. This constant is a simple linear curve fit to manufacture’s free flight data to permit ease of determining velocities at different ranges from the muzzle, but Br is different for each projectile because of the shape and construction of the projectile. Figure 1.2 illustrates the curves fit and Table 1.0 shows the error of the fit is less then 0.5% over the ranges of interest.

![Figure 1.2 Extrapolated data for 9mm and 7.62 NATO Rounds](image)

<table>
<thead>
<tr>
<th>Range (Meters)</th>
<th>9mm Manufacture’s Data (m/s)</th>
<th>9mm Extrapolated Data (m/s)</th>
<th>Range (Meters)</th>
<th>7.62mm Manufacture’s Data (m/s)</th>
<th>7.62mm Extrapolated Data (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>350</td>
<td>0</td>
<td>830</td>
<td>830</td>
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<td>312</td>
<td>309</td>
<td>300</td>
<td>616</td>
<td>614</td>
</tr>
</tbody>
</table>

Table 1.0 Comparison of Extrapolated Data and Manufacture Data
It then follows that the available impact energy (E1) is:

\[ E_1 = \frac{1}{2} m V_1^2 \]  

(equation 2.0)

where \( m \) is the mass of the projectile of interest and \( V_1 \) is the result of equation 1.0, which depends on the range to target.

Since \( E_a \) is the energy dissipated during free flight, which is a function of range to target and projectile design, it follows:

\[ E_a = E_0 - E_1. \]  

(equation 3.0)

\( E_b \) is energy dissipated on impact and depends on whether or not protective gear is used. Without protective gear, the penetration model developed by Sellier (ref a, pages 210-211) through the hair, scalp, skull, and into the brain changes the impact velocity by 110 m/s for the 9mm, or 48.4 joules of energy, which is approximately 10% of the available energy. \( E_b \) maybe represented as a percentage of \( E_1 \) (the energy available at impact) and is computed as:

\[ E_b = (X)E_1, \]  

(equation 4.0)

where "X" is a number much less then 1.0, (0< X<1.0). However this parameter maybe modified if protective gear is used for the different types of projectiles. It follows that the associated magnitude of the velocity (\( V_b \)) is determined by:

\[ V_b = (2E_b /m)^{1/2}. \]  

(equation 5.0)

\( E_i \) is the initial impact energy available in the medium (to form the temporary and permanent cavities and is derived as follows:

\[ E_i = E_1 - E_b, \]  

and

\[ V_i = (2E_i /m)^{1/2} \] its associated velocity.  

(equation 6.0)

(equation 7.0)

During the stable flight portion in the medium, equation (1) is modified to estimate the magnitude of the velocity just prior to the unstable flight. The modified equation simply accounts for the differences in densities between air and 20% gelatine and the difference is a factor of 848:1 changing equation (1) to:

\[ V_2 = V_i - Br (848)(D_1), \]  

(equation 8.0)

where \( D_1 \) is now the distance travelled in the medium prior to becoming unstable, which is observed for the gelatin tests.

The computed energy at this velocity (\( E_2 \)) is:

\[ E_2 = \frac{1}{2} m (V_2)^2 \]  

(equation 9.0)

\( E_2 \) and \( V_2 \) now represent the available energy and associated velocity to form the larger temporary cavity as the projectile tumbles. From the data presented by Bellamy and Zajtchuk
(pages 130 – 131), projectiles typically dissipate 83% of the available energy in the formation of the larger temporary cavity when the flight becomes unstable.

Data also presented by Bellamy and Zajtchuk (page 134) also shows that there is a linear relationship between the maximum diameter of the temporary cavity and the impact velocity, similar to figure 1-3, below. This relationship shows the time to dissipate the available energy in the formation of the large temporary cavity is constant which is true if the medium is homogeneous like gelatin. Though the data shown by Bellamy and Zajtchuk is for soap, the behaviour in gelatin shows the same a linear relationship between maximum radius and velocity, but the specific time required to dissipate the energy is different. These results are from the mathematical model.

![Figure 1-3 Maximum Radius vs. Velocity in Gelatin](image)

Based on the gelatin data depicted below, figures 1-5 and 1-6, dynamic models are developed to depict the formation of the cavities after penetration. These data are obtained from U.S. Army Advanced Research Laboratories(ARL), in a series of reports by Bruchey et al (1979) Specifically, two models are required to capture the dynamics of the cavity formation. One represents the temporary cavity in stable flight after penetration and the other the large temporary cavity during the unstable flight. The size of the permanent cavity is strictly a function of the bullet selected. We have selected a typical 7.62 mm NATO round with a muzzle velocity of 830m /sec and muzzle energy of 3272 j. The 9mm characteristics are: muzzle velocity 350 m /sec; muzzle energy 490 j.

![Figure 1-5 7.62 NATO in Gelatin](image)
Cavity Measurements

<table>
<thead>
<tr>
<th>Penetration Depth (mm)</th>
<th>Cavity (mm)</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>37</td>
<td>20</td>
</tr>
<tr>
<td>66</td>
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<td>94</td>
<td>23</td>
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<td>104</td>
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<td>235</td>
<td>24</td>
</tr>
<tr>
<td>266</td>
<td>22</td>
</tr>
</tbody>
</table>

Figure 1-6 9mm in Gelatin

Dashed outline in both cases shows the large temporary cavity may be approximated by an ellipse, or in three dimensions an ellipsoid, except for different values associated with the major and minor axes of both rounds.

Figure 1-7 depicts the volumes of interest, Volume of the permanent cavity (Vp), the temporary cavity during stable flight (Vs), and the temporary cavity due to unstable flight (Vt).

Figure 1-7 Geometric representations of the permanent and temporary volumes

EMTech Consultants, Inc. Proprietary Information
9
Based on these observations, the calculations for energy and velocities as they relate to the geometric volumes are as follows:

The energy dissipated in forming the temporary cavity is (Ed),

\[ Ed = K3 \times E2. \]  \hspace{1cm} (equation 10)

and K3 is (0.83), representing factor for the dissipated energy.

The volume of the permanent cavity is expressed as:

\[ V_p = \pi (r^2 x), \]  \hspace{1cm} (equation 11)

where \( V_p \) is the volume of the permanent cavity, "r" is the radius of the projectile (\( \frac{1}{2} \) its caliber) and "x" is the penetration distance t.

The volume of the temporary cavity during stable flight is expressed as:

\[ V_{ts} = \pi (r_a^2 D_1), \]  \hspace{1cm} (equation 12)

where \( V_{ts} \) is the volume of the cavity, "r_a" is the cavity radius at penetration and D1 the penetration distance in the medium prior to going unstable.

The volume of the larger temporary cavity caused by the unstable flight is represented by:

\[ V_t = \frac{4 \pi}{3} r_2^2 r_1^2, \]  \hspace{1cm} (equation 13)

where \( V_t \) is the volume of the temporary cavity, "r_2" is the major radius of the temporary cavity and "r_1" is the related minor axis where \( r_1 = ka \times r_2 \). The variables \( r_1, r_2 \) are related to velocity of the bullet within the cavity.

Both models equations (12) and (13) are combined to form the total dynamics of the temporary cavities. However it is noted that the amount of energy dissipated during stable flight is small in comparison to the energy dissipated unstable flight. The geometric representation of equation 13 is our main focus in the development of the mechanical model.

Since \( r_1, r_2 \) are related by, \( r_1 = ka \times r_2 \) and \( ka \) defines the shape of the ellipsoid. Based on the measured data of reference (c), \( ka \) for the 7.62 NATO round has a value of 0.61 and for the 9mm \( ka \) has a value of 0.43. Again, for different rounds there will be modifications to this parameter. It follows that \( r_1 \) and \( r_2 \) are related to the velocities; \( V_{t1} \) and \( V_{t2} \), by:

\[ r_1 = V_{t1}(TM) \text{ and } r_2 = V_{t2}(TM), \]  \hspace{1cm} (equations 14, 15)

where TM is the dissipation time during unstable flight and \( V_{d}^2 = V_{t1}^2 + V_{t2}^2 \).

After the formation of the large temporary cavity, the remaining energy is dissipated in the medium by continuing in flight or comes to rest. The residual energy is \( E_r = E_2 - Ed \). \( E_r \) establishes whether on not sufficient energy remains to exit on the other side of the skull.
The combined volumes are the total volume of interest is represented by:

$$V_{\text{total}} = V_p + V_{\text{ts}} + V_t.$$  \hspace{1cm} \text{(equation 16)}

However, of major interest is the large temporary cavity ($V_t$). This is the focus of our investigations. $V_t$ is compared to the size of the human brain and then scaled down by 672.5:1 for the rat's brain size and designated as $V_{t\text{ rat}}$. The ratio of $V_t$ to $1345 \text{ cm}^3$ is called the Reference Volume ($V_{\text{ref}}$); this number should be much less than one (1) for survival probabilities to increase. In cases where $V_{\text{ref}}$ is equal to or greater than 1 (one), it simply means there is enough energy in forming the large temporary cavity to completely destroy the human brain. The value of $1345 \text{ cm}^3$ is representative of the volume of the human brain, as shown by Walker, A. and Shipman, P., 1996. The volume of the rat’s brain is measured to be $2 \text{ cm}^3$, which establishes the 672.5 to 1 ratio for scaling purposes. The scaling is required to establish the diameter of the probe to be inserted simulating the permanent cavity produced by the projectile.

**Developing A Minimal Invasive Device**

The device for the insertion into the rat’s brain is a probe whose diameter is the scaled diameter of the bullet of interest (7.62mm). The probe contains air holes which are covered by Silex. When air is injected into the probe, by a pneumatic device, the Silex will expand and contract within 30 milliseconds simulating the expansion and contraction of the temporary cavity is produced by the unstable flight portion of the projectile. Figure 2.1 illustrates the design of the probe and Figure 2.2 is a representative prototype to use for the *in vivo* studies. Figure 2.3 shows the Pneumatic device producing the air.

![Illustrative Design of the Probe](image)

*Figure 2.1 Illustrative Design of the Probe*
The final probes for the 7.62 mm round, scaled for the rat are completed. The process for establishing the probe diameter which is representative of the projectile of interest starts with scaling the specific bullet (7.62 or 9mm) using the ratio of 672.5:1 (human brain volume to rat volume) to scale the size of the bullet down to the appropriate lab animal. The scaling assumes the actual bullet is a right circular cylinder and maintains the radius to length to be the same as shown in figure 2.4. Once the diameter is established, this defines the permanent cavity.
of the penetrating wound. The temporary cavity is generated by the expansion of the Silex balloon. The size (volume) of the balloon deployed depends on the amount of brain damage (energy dissipated into the temporary cavity). Since the rat's brain is 2cc in volume, the balloon deployment of 0.2cc represents a 10%, a 0.1 cc would be 5% and 0.3cc would be 15% damage.

To simplify the implementation of the probe and balloon, the length of the ellipsoid representing the temporary cavity was held constant and the diameter was varied to meet the desired volume of damage. The following chart depicts the balloon diameters for a fixed length ellipsoid, for the different damage volumes.

<table>
<thead>
<tr>
<th>Damage %</th>
<th>7.62 NATO</th>
<th>Volume (cm³)</th>
<th>9mm</th>
<th>Volume (cm³)</th>
<th>7.62 AK47</th>
<th>Volume (cm³)</th>
</tr>
</thead>
<tbody>
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<td>25</td>
<td>1.00</td>
<td>0.9525</td>
<td>0.501</td>
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<td>0.9525</td>
<td>0.101</td>
<td></td>
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</tr>
</tbody>
</table>

Since the length of the ellipsoid is held a constant, the balloon sizes for the different rounds would be the same for the same desired percentage damage. This represents a very slight change in the actual geometric shape of the ellipsoid. The reason for this decision was to avoid the necessity of changing probes for the same rounds when an increase (or decrease) in % damage is required. A probe change is always required for the different rounds because the permanent cavities are different.

The pneumatic device is a modified fluid percussion device. It was modified to be a closed air system which simply means when the correct amount of air is (statically) contained in the system,
each time an experiment is conducted the same amount of air remains and the results are repeatable. Figure 2.5 shows a block diagram of the system.

![Block Diagram of PTBI Model](image)

Figure 2.5 Block Diagram of the System.

The performance of the system is based on the weight of the pendulum, the volumetric cylinder and the pressure piston. These components determine the 2psi pressure and the 30msec performance. With the pressure piston manually depressed, the volumetric cylinder is adjusted slowly for the proper (desired) balloon expansion. Releasing the piston after adjustment returns it to its ready (normal) position. The piston is then struck by the pendulum causing the balloon to expand, once stuck the piston returns to its normal position, which brings about the balloon deflation. The entire operation from the time the piston is struck and its return to its normal position is 30 msec.

Since there were no devices in existence to delivery the pneumatic drive, our design performance data was derived from the existing fluid percussion devices. The 30 msec. response time does not reflect the expansion and collapse of the temporary cavity. We need to be down in the microsecond regime to replicate the actual expansion and collapse. However, it has yet to be proven that microsecond performance is required. At this point, further pathology should be conducted to establish design requirements on the mechanical model. The only immediate refinements to this model are to improve the packaging by deleting many of the flexible tubing and make solid connections. To try and improve on this mechanical model really needs a redesign.

The results from the invivo studies are summarized in the following paragraphs.

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We have conducted a series of experiments on rats to determine the efficacy of the Air Inflation Model (AIM) of penetrating traumatic brain injury in modeling the human condition. Several parameters of brain injury were measured, including survival, behavioral outcome and pathology. The air inflation probe was inserted either occipital-frontally (O-F; back-to-front) or left-to-right, and inflated to produce either 0%, 5% or 10% damage to the brain, modeling after the different trajectories and damage produced by projectiles of varying size and velocities. Control animals were subjected to “sham” operations in which the probe was not inserted into the brain.

We had proposed originally to examine 5 different injury paths (i.e. back to front, side to side and oblique angles) as well as 3 different injury levels (i.e. 5%, 10% and 15% balloon); however, we experienced numerous quality assurance difficulties while refining the device. In particular, countless numbers of failed balloon deployments occurred during surgery, as well as balloons bursting upon deployment. Failures in balloon deployment amounted to the use of well over hundred rats. Since we can only assess deployment failures following behavioral and histological analysis of brain sections, this amounted to countless hours of lost time; hence, we could not complete all of the proposed trajectory paths and injury scales as originally proposed. However, once the probe was refined to its “final” form, we were able to examine the front-back and side-side injury paths with up to three AIM injury levels. From our results, we find that the front-back injury paradigm results in a greater loss of survival. As a rule of thumb, we adhered to the local IACUC’s standard of lethality, in which a greater than 50% loss of survival be justified in the continuation of the procedure. The 15% injury level in the front-back lesions resulted in a 30% survival rate after only 7 surgeries. We felt that further experimentation to meet statistical requirement with a 30% survival rate was not justified, so we stopped further experimentation with the 15% balloon level. This result also led us to reexamine the lethality of the obliquely angled lesions as well. Thus, we have completed only the front-back paradigm at the 5% and 10% injury levels and the side to side paradigm at all three levels. As we have had to refine the balloon probe and overcome numerous developmental obstacles, we have not been able to complete a comprehensive anatomical analysis of the injury, including TUNEL staining.

**Behavioral Testing**

Rats surviving the trauma were tested at 1 hr, 24 hr, 48 hr, and 72 hr, post-injury. The scoring is done by two blinded observers. Three scales were used: The Neurological Severity Score (NSS), the Neurobehavioral Scale (NBS) and the NIH Stroke Severity Score (NIH). (Hallenbeck, Dutka et al. 1988)

The NSS was developed in 1988 (Shapira, Shohami et al. 1988), and is a widely used scale in which high scores indicate severe impairment and low scores indicate slight impairment or a normal rat. The maximum score on the NSS is 24. The NSS is scored either 1 (unable) or 0 (able) and examines the ability of the rat to perform in the following tasks. 1) Exit from a circle 50 cm in diameter. The rat is placed at the center of the circle and is given a point if unable to leave before 30 min, another point if it still is unable to leave before 60 min, and a point if unable to leave after 60 min. The first two time intervals of the circle test are only used at the 1 hr test. (Shohami, Novikov et al. 1995) The > 60 min interval continues to be used in the later tests. 2) Righting reflex. A normal rat instinctively rights itself when it is turned over. The recovering
rat is lying on its left (paretic) side. A point is assigned if the rat is unable to right itself 20, 40 and/or greater than 60 min after injury. The first two time intervals are only used at the 1 hr test. The last interval is still used in later tests. 3) Hemiplegia. The rat is pushed back and forth at the shoulders and should resist equally in both directions. A point is given if resistance is not equal. 4) Hind limb flexion. A normal rat when raised by the tail will extend both hind limbs, reaching upwards. If the rat flexes a hind limb, a point is given. 5) Walk in a straight line and ability to move. A point is allotted for each function. 6) Startle reflex to a loud noise about 20 cm above the rat’s head. (Germano, Dixon et al. 1994) The rat should flinch heavily. If it does not, a point is given. 7) Pinna reflex to touching the external auditory meatus with a cotton tipped ear swab. (Chen, Li et al. 2001) If the rat does not shake its head back and forth, a point is assigned. 8) Seeking behavior and ability to stand. If a rat has lost its seeking behavior (a normal rat will walk around and sniff unknown objects), the rat receives a point. The point is only given if the rat would have been graded a 2 (moderate impairment) or lower on the corresponding category of the NBS. If the rat is prostrated, another point is given. 9) Placing reflexes. The rat is lifted 5 cm off the ground by the tail and back. The animal should “reach” for the ground and place its limbs on the floor with palms facing the ground. A point is allotted for each limb’s inability to place. 10) Balance beam and beam walking. The rat is placed on a 1.5 cm wide beam. A point is given if the rat falls off within 60 sec, another point if that fall was within 40 sec, and another point if that fall was within 20 sec. The rat is then placed on beams 2.5 cm, 5.0 cm, or 8.0 cm in width. A point is given for failure on any width.

The NBS is divided into four categories. Rats are graded on a scale of 0-4, with 4 being normal and 0 being non-functional. The first category is forelimb flexion upon suspension by tail. A normal rat will extend both forelimbs and reach for the ground. (Bederson, Pitts et al. 1986) A “4” is given for normal forelimb extension, a “3” for slight forelimb flexion, a “2” for moderate forelimb flexion, a “1” for severe forelimb flexion, and a “0” if the forelimbs are tucked in next to the body. The second category is decrease in resistance to lateral pulsion. The rat is pulled by each limb. Resistance should be equal in both directions. A “4” is given for normal a “3” for slight impairment, a “2” for moderate impairment, a “1” for severe impairment, and a “0” for no resistance at all. The third category is circling behavior upon spontaneous ambulation. A normal rat, when placed on the floor should be able to walk straight. If the rat is able to walk straight even if it does show partial circling, it is given a “4”. If the rat always walks to the paretic side, it is given a “3”. If the rat shows partial circling and always walks to the paretic side, it is given a “2”. If the rat circles when attempting to walk, it is given a “1”. If the rat can only spin in place, it is given a “0”. (Reglodi, Somogyvari-Vigh et al. 2000) The fourth category is ability to stand on an inclined plane. The rat is placed on an 8.0 cm wide board at a specified angle. The rat is given a “4” if it can stand on the 45°-50° board, a “3” if it can stand on a 40°-45° board, a “2” if it can stand on a 35°-40° board, a “1” if it can stand on a 30°-35° board, and a “0” if it cannot stand on the 30° board. The final category of the NBS is open-field activity/exploratory behavior. The rat is placed on the floor and observed. A normal rat should explore the area and sniff unknown objects. A normally exploring rat is given a score of “4”. A rat that sniffs and explores, but not to a normal degree, a “3” is given. If the rat either does not sniff or does not explore at all, a “2” is given. If the rat neither sniffs nor explores a “1” is given. If the rat does not move, a “0” is given.

The final scale is the NIH scale. A grade of 0 is given to a normal rat. A grade of 1 is given to a lethargic rat. A grade of 2 is given to a rat with clear signs of paresis, but with the ability to walk. A grade of 3 is given to a rat with the inability to walk. A grade of 4 is given to a dead rat. (Hallenbeck, Dutka et al. 1988)
Pathology

Following neurological testing on day 3, rats were perfused transcardially with 0.1M PBS followed by 10% formalin solution. Brains were removed from the cranium and embedded into paraffin. Coronal (medial-lateral) and sagittal (rostral-caudal) sections were cut on a microtome, deparaffinized and stained with either hematoxylin and eosin, or 0.5% cresyl violet solution. Sections were coverslipped and observed using a microscope. Cells were counted in 3 separate high power fields along the probe track (injured group) or at similar anatomical sites (sham group) by 2 different observers blinded to the animal’s treatment. The mean of both observers and the 3 fields was recorded. Similar cell analysis was performed in the hippocampus ipsilateral to the probe track (injured group) or at a similar anatomical site (sham group).

Statistical Analysis

A Kruskal-Wallis One Way ANOVA on Ranks test with Tukey or Dunn’s post-hoc analysis was performed on all behaviorally scored data.

Results

Survival following PTBI

Survival of animals subjected to sham, O-F and left-right operations are summarized in Table I. All of the sham-operated animals survived the surgery and the subsequent behavioral testing procedures, while several animals in either the O-F and left-right PTBI groups died following surgery.

Table I: Survival of Rats Following Penetrating Traumatic Brain Injury

<table>
<thead>
<tr>
<th>Group</th>
<th>Occipital-Frontal</th>
<th>Left-Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>0% Balloon (injury)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>5% Balloon (injury)</td>
<td>100%</td>
<td>91%</td>
</tr>
<tr>
<td>10% Balloon (injury)</td>
<td>62%</td>
<td>91%</td>
</tr>
<tr>
<td>15% Balloon (injury)</td>
<td>30%</td>
<td>71%</td>
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</tbody>
</table>

Increasing balloon size resulted in the increased number of deaths, especially in the O-F group, where there was a substantial increase in fatalities when the balloon size was doubled from 5% to 10%. Doubling the volume implies more energy is dissipated into the brain to produce a 5 to 10% increase in the damage volume, but energy is not a linear function of the volumetric size. Since increasing the balloon size in the O-F group to 10%, resulted in a near 50% survival rate, we chose not to increase the balloon size to 15%. The survival rates suggest that injury size is not the only determinant in life or death outcomes, but more importantly, the trajectory or route of the injury.
Behavioral Testing

Animals surviving surgery were functionally examined using three different neurological tests. Sham-operated control and probe only (no balloon) animals exhibited little/no functional deficits as a result of O-F surgical procedures on the NSS, NIH and NBS behavioral tests, while 5% and 10% balloon groups exhibited significant (p<0.05) behavioral deficits when tested 1 hr following surgery on all of these tests. The 5% and 10% PTBI animals were also impaired at the latter time points tested and did not recover to control levels prior to sacrifice (Figure 1). Specific tasks in which the PTBI animals had performed poorly included, walking in a straight line where lesioned animals walked in circles, forepaw and hindlimb flexion on the side opposite the lesion when lifted by the tail, and hemiplegia towards the side opposite the lesion.

Left-right PTBI animals exhibited significant neurological deficits (p<0.05) one hour following injury. The NSS, NIH and NBS test results showed significant differences between the sham group and the 10% and 15% balloon groups one hour after injury. Moreover, the 15% group was significantly impaired on the NSS when tested 48 hrs after injury (Figure 2). As with the O-F lesioned groups, the left-right animals continued to show impairment on all neurological tests up to three days post-injury with some degree of recovery, but never to control levels. In contrast to the O-F group, animals in the left-right groups preferentially showed disturbances in balance and bilateral forepaw flexion when lifted by the tail.

Left-right lesioned animals scored higher (performed poorly) on the NSS tests and were better performers on the NBS tests in contrast to their O-F lesioned counterparts, that performed poorly on the NBS and scored lower (performed better) on the NSS tests. Since the NSS test is designed to assess reflexes and basic motor skills, poor performance on this test suggests bilateral damage to the reflex centers of the brain, which would abolish and/or diminish the reflex. On the other hand, O-F lesioned animals experienced difficulty in behavioral tasks such as movement and posture which suggests damage limited to one side of the brain. Moreover, increase in balloon size resulted in decreased performance in the behavioral tasks tested in both left-right and O-F groups, which support the survival data.
Figure 1. Effect of occipital-frontal lesions on the NSS, NIH and NBS behavioral assessments. Sham-operated animals suffered little/no functional deficits compared with 5% and 10% balloon PTBI animals.
Figure 2. NSS, NIH and NBS behavioral test results from sham-operated and 5%, 10% and 15% left-right-lesioned animals. Sham-operated animals exhibited little/no behavioral deficits upon testing, while the lesioned animals exhibited neurological deficits.
Histopathology

Hematoxylin and eosin (H&E), and cresyl violet-stained sections of brains from PTBI animals revealed extensive neuronal death and gliosis in O-F and left-right sections. Examples of left-right lesions are depicted in Figures 3 and 4. Examples are from 10% balloon PTBI animals.

Figure 3 Hematoxylin and eosin-stained sagittal section of a left-right 10% balloon PTBI animal. Arrows point to the lesion created by the balloon.

Figure 4 High power magnification of lesion site. Black arrows delineate the outermost extent of the lesion (prenumbra), while the white arrowheads mark the central area of the lesion occupied by the pale scar.
Examples of occipital-frontal lesions are depicted in Figure 5. Examples are from 10% balloon PTBI animals. Cell counts of the sham-operated and probe only (0% balloon) groups are depicted in Figure 6 for necrosis and Figure 7 for apoptosis (as measured using TUNL staining).

Occipital-frontal lesioned animals exhibited extensive damage on H&E stained sagittal brain sections with enlarged ventricles (Figure 5), which were not observed in left-right lesioned animals. The enlarged ventricle suggests increased cellular loss and/or damage, which could explain the behavioral and/or survival results.

Figure 5  O-F 10% PTBI lesioned animal. Arrows point to the lateral ventricle. Note the difference in the size of the lateral ventricle in the O-F lesioned animal when compared with the 10% left-right lesioned animal (Figure 3).

Figure 6. Cellular necrosis in peri-injury site and ipsilateral hippocampus. Probe with 0% balloon demonstrated significantly greater cellular injury than sham-operated animals. There was virtually no injury to hippocampus.

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Figure 7. Apoptosis in peri-injury site and ipsilateral hippocampus. Probe with 0% balloon demonstrated significantly greater apoptotic cell damage than sham-operated animals. Although there was injury to the hippocampus, this was no significantly different from sham-operated animals.

Insertion of probe, even without balloon inflation, resulted in significant cellular necrosis and apoptosis in peri-injury site along the probe track. However, there was little damage noted in ipsilateral hippocampus which is anatomically remote from this injury path (Figs. 6 & 7).

**TUNEL labelling of balloon in injured animals**

In order to determine the extent of apoptosis or programmed cell death following PTBI, we labeled apoptotic cells using the TUNEL method. Examples of typical results are shown in Figure 6. TUNEL-labeled cells were most frequently observed at the entry of the probe and at the end of the probe (exit), but not at the dorsal or ventral boundaries of the lesion. Few labeled cells were observed in structures like the hippocampal formation, which on gross pathological examination, was not affected by the lesion in the side to side trajectory, as it was posterior to the probe. On the other hand, the cerebellum exhibited numerous labeled cells on back to front lesions, as the probe entered the brain through this structure.

Preliminary cell counts of TUNEL-labeled cells were conducted on the dorsal hippocampus on viable sections of 10% front-back lesioned animals (the left-right lesions damaged the dorsal hippocampus where counts were conducted, so no counts were obtained). Results are summarized in Figure 6. There was no statistically significant changes between sham-operated and injured animals in the number of TUNEL-labeled cells (n=5), suggesting that damage produced by the probe passing through the fimbria-fornix (see Figure 5) did not injure the dorsal hippocampus, at least after 3 days of survival.

Recently, studies have shown that the methodology used in the TUNEL labeling may be non-specific as it also labels dividing cells (Pulkkanen et al., 2000), necrotic cells (Garrity et al., 203), as well as exhibiting false positive staining in response to proteinase K treatment (Stahelin et al., 1998) and histological sectioning (Sloop et al., 1999), the latter two methods having been used in our studies. To rectify the situation, we are currently employing immunohistochemical techniques to identify apoptotic cells using antibodies to cleaved caspase -3 (Brecht et al., 2001).
Figure 6. Photomicrographs of DAPI (nuclear) and TUNEL (apoptotic) cells in the cerebellum (upper panel) and hippocampus (lower panel) of PTBI brains.
To summarize, the two different PTBI models (left-right and occipital-frontal) produces extensive neuronal damage to the brains of rodents resulting in decreased survival, with each type of injury producing unique functional deficits. Histological examination of brains reveals gliosis and scar tissue formation at the site of the lesion with alterations in ventricular size, suggesting neuronal death and/or injury. These results support and validate the air-inflation model of PTBI as a "tool" by which one can examine the role of penetrating objects on neuronal integrity and the ensuing functional outcome following brain injury.

Key Research Accomplishments

The mathematical model is complete and maybe used as intended, to predict the size and shape of the temporary cavities for different impact energies and velocities associated with the 7.62 NATO round. This mathematical model is the first in the investigation of PTBI.

The mechanical model has been calibrated and used laboratory in vivo studies. The in vivo studies will serve to validate the mathematical / mechanical predictions.

The in vivo studies provide the data which validates this model and its use as a PTBI tool by which one can examine the role of penetrating objects on neuronal integrity and the ensuing functional outcome following brain injury.

Reportable Outcomes

Included are sample outcomes of the mathematical PTBI model. Based on these outcomes the mechanical probe was designed and in vivo studies were conducted on the rat specifically using the 7.62 NATO round. For other laboratory animals the mathematical will have to be re-scaled for the different animals and a new probe will have to be developed to be representative of the specific ammunition used.

Sample calculations for the 9mm predicting volume size and shape at various ranges:

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<thead>
<tr>
<th>9mm</th>
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</thead>
<tbody>
<tr>
<td><strong>INPUTS</strong></td>
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<td>mass (g)=</td>
</tr>
<tr>
<td>Muzzle velocity (m/s) (Vo)=</td>
</tr>
<tr>
<td>Muzzle energy (joules) (Eo)=</td>
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<tr>
<td>Range to target (m) (Rm)=</td>
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<tr>
<td>Coupling Coefficient Ka Ka=</td>
</tr>
<tr>
<td>Diameter (caliber) (cm)=</td>
</tr>
<tr>
<td>Vel/Range Coef. Br : Br=</td>
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<tr>
<td><strong>Assumptions</strong></td>
</tr>
<tr>
<td>Energy Diss on Impact Eb (j) Eb=</td>
</tr>
<tr>
<td>Density of (gelatine/air)=</td>
</tr>
<tr>
<td>Dissipation into Temp Cav K3. =</td>
</tr>
</tbody>
</table>

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| Dissipation time (sec) TM | 0.000411 | 0.000411 | 0.000411 | 0.000411 | 0.000411 | 0.000411 |
| Dissipation time (Sec) TM2 | 0.000488 | 0.000488 | 0.000488 | 0.000488 | 0.000488 | 0.000488 |

### Computing Energy & Velocities

#### Energies

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<td>Energy at range (j) E1</td>
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<tr>
<td>Energy Diss in Free Flt EA (j) Ea</td>
<td>18.85054, 37.34016, 115.9454, 153.2287, 191.5345, 243.984</td>
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<td>Energy Diss on Impact Eb (j) Eb</td>
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<td>Energy Avail at medium Ei (j) Ei</td>
<td>343.34496, 324.8598, 246.2546, 208.9713, 170.6655, 118.216</td>
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<td>Energy Diss Stable Ec (j) Ec</td>
<td>93.88422, 91.11779, 78.36407, 71.59893, 63.98435, 51.99751</td>
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<tr>
<td>Energy Diss in Ig cavity Ed (j) Ed</td>
<td>207.052414, 194.0059, 139.3491, 114.0191, 88.54539, 54.96135</td>
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<td>Residual Er (joules) Er</td>
<td>42.4083258, 39.73615, 28.54138, 23.3533, 18.1358, 11.25714</td>
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#### Velocities

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<td>292.978224, 284.9824, 248.1202, 228.5669, 206.5584, 171.9128</td>
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<td>249.730224, 241.7344, 204.8722, 185.3189, 163.3104, 128.6648</td>
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<tr>
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<td>Computing Vd (m/s) Vd</td>
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<tr>
<td>Computing Vr (m/s) Vr</td>
<td>102.966409, 99.66964, 84.47098, 76.40894, 76.40894, 67.33462</td>
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#### Dimensions - Low Speed

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<td>89.8747633, 86.99716, 73.73093, 66.69394, 58.77337, 46.30483</td>
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<td>Computing Vt2 (m/s) Vt2</td>
<td>209.011077, 202.319, 171.4673, 155.1022, 136.6823, 107.6857</td>
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<td>Computing D1 (cm) D1</td>
<td>7.4762898, 7.365316, 6.830434, 6.528946, 6.172011, 5.563923</td>
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<td>Computing R1 (cm) R1</td>
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<td>Computing R2 (cm) R2</td>
<td>8.59035528, 8.31531, 7.047305, 6.3747, 5.671641, 4.4258</td>
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#### Volumes

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<td>Volume Ig temp (cm³) Vt</td>
<td>491.038117, 445.3661, 271.1134, 200.6601, 137.3231, 67.15538</td>
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<tr>
<td>Volume Stable Cavity Vca</td>
<td>146.015641, 144.6364, 134.1326, 128.2122, 121.2029, 109.2615</td>
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<tr>
<td>Total Volumes Vt + Vca (cm³)</td>
<td>637.853758, 590.0025, 405.2461, 328.8723, 258.526, 176.4169</td>
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<td>Total RAT Volume (cm³) Vrat</td>
<td>0.94848142, 0.907696, 0.623456, 0.490597, 0.397732, 0.271411</td>
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#### Rat Vol from Vt/672.5(cm³) Vrat1

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<tr>
<td>0.7301682, 0.662254, 0.403143, 0.298379, 0.204198, 0.099859</td>
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#### Compute Rat Rr2 (cm) Rr2

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<td>0.68266728, 0.66081, 0.560043, 0.506591, 0.446428, 0.35172</td>
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#### Ref Volume Ig temp (Vt) / 1345

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#### Reference Total Volume / 1345

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#### Reference Volume Rat

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Sample calculations for the 7.62 NATO predicting volume size and shape at various ranges:

### 7.62 NATO

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<td>0.72</td>
<td>0.72</td>
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**Assumptions:**
- Density of (gelatine/air) = 848 848 848 848 848 848
- Dissipation into Temp = 0.83 0.83 0.83 0.83 0.83 0.83
- Dissipation time (sec) TM = 0.00028 0.00028 0.00028 0.00028 0.00028 0.00028
- Dissipation time (Sec) TM2 = 0.000406 0.000406 0.000406 0.000406 0.0004059 0.000406

**Computing Energy & Velocities**

**Energies**
- Energy at range (j) E1 = 3131.884 2994.571 701.5838 627.4218 541.11881 431.843
- Energy Diss in Free Fit EA (j) Ea = 140.116 277.429 2570.416 2644.579 2730.8812 2840.157
- Energy Diss on Impact Eb (j) Eb = 127.8 127.8 127.8 127.8 127.8 127.8
- Energy at medium Ei (j) Ei = 3004.084 2866.771 573.7838 498.621 413.31881 304.043
- Energy Dist Stable Ec (j) Ec = 652.0733 636.075 262.5509 242.3326 216.8074 180.281
- Energy avail for Ig Cavity E2 (j) E2 = 2352.011 2230.696 311.2329 257.2884 194.8172 123.762
- Residual Er (joules) Er = 1952.169 1851.478 258.3233 213.5494 163.10446 102.7224
- Energy avail at medium Ei (j) Ei = 399.8418 379.2183 52.90959 43.73903 33.406937 21.03954

**Velocities**
- Vel at range V1 (m/s) V1 = 812 794 384.32 363.44 337.52 301.52
- Computing V1 (m/s) V1 = 795.2602 776.8724 347.5581 324.3199 294.98218 253.00001
- Computing V2 (m/s) V2 = 703.6762 685.2884 255.9741 232.7359 203.39818 161.4161
- Computing Vc (m/s) Vc = 370.5112 365.9379 235.104 225.8703 213.64379 194.8172
- Computing Vd (m/s) Vd = 641.0795 624.3275 233.2035 212.0325 185.30456 147.0571
- Computing Vr (m/s) Vr = 290.1331 282.5516 105.5408 95.95945 83.863219 66.55356

**Dimensions-Supersonic**
- Computing Vt1 (m/s) Vt1 = 333.848 325.1243 121.4428 110.4179 96.499056 78.56132
- Computing Vt2 (m/s) Vt2 = 547.2919 532.9906 199.0866 181.0129 158.19517 125.5431
- Computing R1 (cm) R1 = 9.347745 9.103479 5.574426 5.06836 4.4294649 3.515208
- Computing D2 (cm) D2 = 15.32417 14.92374 5.574426 5.06836 4.4294649 3.515208
- Computing Vt (m/s) Vt = 5609.649 5181.283 270.0256 202.9584 135.47475 67.71076
- Total Volumes Vt + Vca (cm3) Tot Vol = 5904.979 5472.967 457.4238 382.95965 305.76725 222.99722
- Tot RAT Volume (cm3) Vrat = 8.780637 8.138241 0.680184 0.569512 0.4546725 0.331594
- Rat Vol from Vt / Vt2 672.5(cm3) Vrat = 8.341486 7.70451 0.401525 0.301797 0.2014494 0.100685
- Compute Rat Rr2 (cm) Rr2 = 1.94114 1.890416 0.706122 0.642018 0.5610881 0.445278

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Compute Rat Rr1(cm) Rr1=

| Reference Total Volume/1345 | 4.390319 | 4.069121 | 0.340092 | 0.284756 | 0.2273362 | 0.165797 |
| Reference Volume =V/1345    | 4.170743  | 3.852255 | 0.200763 | 0.150898 | 0.1007247 | 0.050343 |
| Reference Volume Rat         | 4.170743  | 3.852255 | 0.200763 | 0.150898 | 0.1007247 | 0.050343 |

Conclusions- All objectives of this study have been met. To reiterate these objectives:

The initial objective is to develop a mathematical model for rats that describes the biophysics of wound formation following a ballistic injury. The bullet-size model will be the full metal jacket ammunition like the 7.62mm round and the 9mm which are most commonly used in the military. These equations can then be used generically to determine the dimensions of the salient features of ballistic injury, i.e. the size and shape of the permanent and temporary would cavities, rapidity of expansion and duration of inflation. This is completed and sample results provided

The second objective is to develop a device for minimal invasive recreation of the wound in rats. This device will be based on an air inflation technique. It will be built exactly to precise specifications based on the above mathematical model. This devise will be designed in accordance with all elements of the wound arising from a gunshot. The wound cavities assume the proper size, shape and for the appropriate duration. This is completed and specifically developed for the 7.62 NATO round appropriate for the laboratory rat.

The third objective will be to conduct in vivo validation study in rats to demonstrate that this model can recreate wounds similar to those caused by military gunshot. Histopathologic findings will be compared to the results from human gunshot victim autopsy reports and to the work of Carey et al (1989, 1990) using the now-abandoned fired projectile feline model. This has been completed and the in vivo studies support and validate the air-inflation model of PTBI as a “tool” by which one can examine the role of penetrating objects on neuronal integrity and the ensuing functional outcome following brain injury.
References


3) Sellier and B.P. Kneubuehl, "Wound Ballistics and the Scientific Background", 1994 Chapter 4, Ammunition and Arms, Ballistics, Pages 111 to 123.


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MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for Grant DAMD17-01-1-0742. Request the limited distribution statement for Accession Document Numbers ADB287012 and ADB292823 be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@amedd.army.mil.

FOR THE COMMANDER:

[Signature]

PHYLIS M. RINEHART
Deputy Chief of Staff for Information Management